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Study of the Effects of Eucalyptus and Lavandula Essences on the Growth of *Candida albicans* Strains Resistant and Sensitive to Itraconazole in Iran

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As *Candida albicans* have grown more resistant to anti-fungal agents , and also with respect to the restrictions in treatment of fungal diseases studying combinations such as herbal medicine which could replace them seems essential. The aim of the present study is to investigate the effects of herbal essences of eucalyptus and lavandula on the growth of clinical *Candida albicans* strains and compare them with those *Candida albicans* which are resistant and sensitive to itraconazole. The present study has been carried out on over 41 *Candida albicans* taken from patients suffering from candidiasis. The degree of samples' sensitivity to eucalyptus and lavandula were determined through applying broth microdilution method.In the present study, the MIC of eucalyptus against *Candida albicans* was determined at 4-1024µl/ml, in which the most growth fluctuations it had were seen in the densities of 64 and 32µl/ml. Also, the MIC of lavandula was determined at 1-256 µl/ml, and the most fluctuations it had in its growth were in the densities of 32 and 16µl/ml. Eucalyptus and lavandula essences have a very good anti-fungal effect against *Candida albicans*. In the present study, lavandula essence proved better anti-candidiasis effects while no considerable difference was seen between the strains sensitive and resistant to itraconazole in its MIC. In higher densities, eucalyptus essence prevents strains from growing.

Key words: *Candida albicans*, Eucalyptus , Lavandula, Essence, MIC, Itraconazole.

Studies conducted from 1980 onward have shown that the systemic and opportunistic infections resulting from Candida have been increased significantly. In spite of the fact that there have been too many pathogenic Candida species responsible for candidiasis infections, one of the most important and most pathogen species of all is albicans[1]. *Candida albicans* is the natural yeast of Mucosa and skin, which is limited to skin in the form of saprophyte. The range of this infection varies from mucosal colonization to aggressive and lethal infections. It can easily turn into pathogen and cause mucosal, cutaneous and - in uncommon cases - systemic diseases in case of having proper environment, weak immunity system, taking systemic corticosteroids and antibiotics, and in malignity[2].Azole antifungal drugs such as itraconazole are the main stay of therapy in the management and prophylaxis of candidiasis. However,in recent years,itraconazole-resistant clinical isolates of *Candida albicans* have emerged in Iran.

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Generally speaking, treatment of candidiasis is very complicated as candidiasis strains resistant to common anti-fungal agents are formed. Besides being limited in their amount, most common anti-fungal agents are also very expensive. Recurrence of candidiasis infections is very common, and this adds to the importance of treating this opportunistic infection and consequently has raised the need for developing new anti-fungal agents for expanding the spectrum of anti-candida activities and combating against strains resistant to available anti-fungi[3].

For decades, humans have discovered properties of herbs and have used them since then, but it was only after 19th century when chemical combinations replaced herbal medicine. However, scientists now a days pay particular attention to using herbal medicine and traditional medicine methods. In countries like Iran remarkable history could be traced about applying herbal medicine for treating diseases; For instance great Iranian scientist Abu Ali Sina (Avicenna) had considered using herbs like lavandula[4-7]. Considering the fact that many countries enjoy too many herbal strains which have numerous medical properties and also as senior officials have emphasized on applying traditional and herbal medicines, using such herbs for treating infections and preventing medical resistances seems quite reasonable.

The present study aims to determine the MIC (minimum inhibitory concentration) of eucalyptus and lavandula essences in preventing the growth of *Candida albicans* separated from patients suffering from mucosal, cutaneous and visceral candidiasis. Also, those *Candida albicans* resistant and sensitive to itraconazole will also be studied in terms of their sensitivity to the aforementioned essences to see whether or not strains resistant to itraconazole are also resistant to these essences as well .

MATERIALS AND METHODS

In the present study, 41 *Candida albicans* isolates including 23 isolates taken from mucosal infections, 16 isolates from cutaneous infections, and 2 isolates from visceral infections in five hospitals in Tehran(central region of Iran) , Gorgan and Sari cities(northern parts of Iran)were collected.These strains were studied to find and verify albicans species through direct test, culturing over corn meal agar containing tween 80, germ tube test, colony color on Chromagar, and the test of glucose absorption by API120 kit. In the present study, *Candida albicans* (A90029) was used as the standard strain.

For determining the sensitivity or resistance to itraconazole anti-fungal agent, broth micro dilution method was applied. According to the instructions proposed by CLSI, strains with the MICd"0/125µg/ml are considered sensitive and the ones with MIC≥1g/ml are considered resistant to itraconazole[8]. In the present study, 32 strains were reported as sensitive to itraconazole, and 9 strains as resistant to itraconazole.

In order to determine the sensitivity of the *Candida albicans* strains toward eucalyptus and lavandula essences, first a suspension of 48-hour *Candida albicans* strains cultured in saburo dextrose agar with distilled water was prepared. The initial sample containing 10^6 yeasts as per each CC of distilled water was then prepared by spectrophotometer in the wavelength of 530 nanometers and with transition of 75-77. After that, the sample was diluted first at the ratio of with distilled water, and finally at the ratio of with RPMI 1640 culture medium (with glutamines, without bicarbonate, and with PH indicator of Sigma Co.) and MOPS buffer (Sigma Co.) so that the final number of yeasts reaches10^3cfu/ml.

Eucalyptus and lavandula essences were supplied from Barij Essence Pharmaceutical Company. Dimethyl sulfoxide(DMSO) was used for making stocks; therefore, 1.024 milliliters of eucalyptus essence was dissolved in 4 milliliters of DMSO and 0.3 milliliters of lavandula essencewas also dissolved in 4.7 milliliters of DMSO to reach the final 5 milliliter volume.

All the stocks were diluted at the ratio of with the RPMI. Then, 0.1 milliliter of each stock was mixed with 9.9 milliliters of RPMI medium in the tube, and 100 microliters were inseminated in the micro plate wells containing 100 microliters of RPMI medium and were then diluted serially. After that, 100 microliters of the yeast suspension with the density of 10^3cfu/ml was then inseminated in each well. RPMI combination and yeast were used as the positive control in one well, and RPMI and essence were used as the negative control.

For investigating the anti-candidiasis

effects of eucalyptus essence, the densities of 4-1024 µl/ml and densities of 1-256 µl/ml were also used for lavandula essence. Micro plates were incubated for 48 hours in 35º C after being capped, and were then studied in terms of their growth after the incubation time was over. In cases in which no growth was observed in the well containing negative control, wells were checked with special mirrors to reassure that the process has been done correctly.

RESULTS

In the present study, the average MIC of eucalyptus against *Candida albicans* has been determined at 50 µl/ml in total (from among 4-1024), in which most growth fluctuations were observed in densities of 64 and 32. In the density of 256 in all organisms, no growth was observed, while growth was seen in all other densities without any change(Table1). From among clinical strains, only cutaneous strains were found to be sensitive to the densities of lower than the essence (128µl/ml). The average MIC of eucalyptus against the standard *Candida albicans* was determinedat 128 µl/ml. The average MIC of eucalyptus against those *Candida albicans* resistant to itraconazolewas determined to be 128µl/ml and that of *Candida albicans* sensitive to itraconazole was reported to be 64µl/ml, which proves that there is no considerable difference between strains sensitive and resistant to itraconazole (Table3).

The average MIC of lavandula against *Candida albicans* was reported at 64 µl/ml (from among 1-256), in which the most growth fluctuations were seen in densities of 32 and 16. No growth was also observed in the densities of 256, 128 while growth was observed in all other

Table 1. Comparison of growth or no growth of *Candida albicans* strains in the densities of 64,32 µl/ml eucalyptus essence

essence /	strain	no growth		growth		Comparison
Density	1×10^3cfu/ml	number	percent	number	percent	
64	*C.albicans*	29	70/7	12	29/3	x^2=0NS
32	*C.albicans*	5	12/2	36	87/8	x^2=0NS

NS:Not significant

Table 2. Comparison of growth or no growth of *Candida albicans* strains in the densities of 32,16 µl/ml Lavandula essence.

essence /	strain	no growth		growth		Comparison
Density	1×10^3cfu/ml	number	percent	number	percent	
2	C.albicans	34	82/9	7	17/1	x^2=0NS
16	C.albicans	25	61	16	39	x^2=0NS

NS:Not significant

Table 3. Quantative distribution of Minimum Inhibitory Concentration in two herbal essences according to their sensitivity to itraconazole

MIS/ essence	*C.albicans* Resistant or sensitive to itraconazole	Average	Standard deviation	comparison
Eucalyptus	Resistant	128	18	NS
	Sensitive	64	15.2	NS
Lavandula	Resistant	64	7.2	NS
	Sensitive	32	6.5	NS

NS:Not significant

densities with no change (Table2). The average MIC of lavandula against the standard strain of *Candida albicans* was determined at 16µl/ml.From among clinical strains, mucosal and cutaneous ones showed most sensitivity in the density of 32µl/ml.

The average MIC of lavandula for the strains sensitive to itraconazole was determined to be 32 and for the strains resistant to itraconazole was determined at 64, which again proves no considerable difference between the strains sensitive and resistant to the antibiotic(Table3).

DISCUSSION

Azoles destroy fungi by disordering the synthesis of fungal ergosterol. Thanks to theirwide range of usage, these agents are among highly-consumed drugs in treating clinical cases of fungal infections[9,10]. The mechanism of these drugs is toleave significant side-effects on the host body. Studies conducted since 1999 prove that *Candida albicans* which cause infections in human beings and animals are becoming more resistant to drugs, especially to azoles[11]. This problem is particularly seen in treating candidiasis infections using itraconazole, fluconazole, and clotrimazole. Epidemiologic studies have shown that major fungal infections are mainly caused by species resistant to anti-fungal agents. This is highly emphasized in case of the effects itraconazole anti-fungal drug have on Candida species[12].

In a study conducted in 2013, Hasna Boura *et al.*, found out that from the 56 *Candida albicans* strains separated from clinical samples (the dominant strain), 12.4% were resistant to itraconazole[13]. Also, in studies Badiee conducted over 595 yeast strains separated from clinical samples in 2011, 48% were found to contain *Candida albicans* with the average MIC of 4 µl/ml for itraconazole, proving that these strains are becoming more resistant to itraconazole[14].

Taking the above into consideration, the tendency to find new antifungal combinations to replace the existing drugs is growing. Thus, it is important to conduct more studies on these herbs so as to discover more effective medicines and consequently better treatments.

Eucalyptus tree, which is called with its scientific name, was imported to Iran over fifty years ago. These trees were first planted in southern parts and then in the northern parts of the country; as the oldest ones nowadays are found in Fars province in southwest Iran. Eucalyptus species mostly grow in semi-arid and sub-humid climates.Eucalyptus leaves, especially those of older trees, are the main parts of this plant to be used. These leaves contain essences and oils with antimicrobial effects[15]. The essence of eucalyptus is comprised of cineol (70%-85%); but it also contains monoterpenes(such as linalool and borneol), and also sesquiterpenes such as beta - caryophyllene[16-19]. In the present study, the MIC of eucalyptus essence was found within the range of 4-1024 µl/ml, and no growth was seen by *Candida albicans* strains resistant to itraconazole in densities higher than 256.

Takahashi *et al.*, studied antimicrobial effects of eucalyptus in 2004, and found three components existing within eucalyptus essence to have positive antifungal effects in treating skin infections[17].

In their study in 2012, Kottearachchi et al found out that the components existing in eucalyptus oil has antifungal effects on grains toxic-causing fungi like Fusarium[20]. In a study conducted by Bokaeian, Eucalyptus globulus was proved to have positive anti-candidiasis effects in diabetic mice infected with candidiasis[21].

In the present study, the average MIC of eucalyptus against resistant *Candida albicans* was found to be 128, and the one for *Candida albicans* sensitive to the antibiotic was found to be 64. As no significant difference was found between strains resistant and sensitive to itraconazole, it could be concluded that eucalyptus essence could be a good replacement for chemical drugs by determining the numeric value of MIC.

Lavandula belongs to the Lamiaceae family and could be easily planted almost anywhere in the world including Iran. Most Iranians are quite familiar with this plant, and frequently use it in traditional medicine and herbal therapy.

This short-lived plant has thin long green leaves, and its essence which is taken through distillation of its flower and the flowering branches, is a yellow or a greenish yellow with a pleasant odor[22]. Its essence contains about 40% linalyl acetate, as well as butyric acid, propionic acid, valeric acid, free linalool, and geraniol.

In the present study, the MIC of lavandula was determined within the range of 1-256 µl/ml, and no growth was seen among strains resistant to itraconazole in densities higher than 256.

Other studies conducted in 2005 and 2011 showed that essential oils and essence combinations existing in lavandula have very high fungicide effects on the yeast and germ tube forms of human isolates of *Candida albicans*[23,24]. The antifungal effects of lavandula essence and essential oils against *Candida albicans* were studied by Millak et al, and they found Linalool as the most effective combination in this plant[24].

The results of the present study prove the anti-candidiasis effects of lavandula essence. Moreover, with regards to the results gained by comparing the MIC of strains resistant to itraconazole and the ones sensitive to it, it could be concluded that lavandula could be used in treating candidiasis infections and treatment-resistant candidiasis in particular.

As the anti-candidiasis effects of the studied essences have been proved and no resistance has been seen to these essences, and also due to the fact that Candida is growing more medical resistance, using such plants seems quite reasonable, not mentioning that the climatic conditions of many countries are also well enough for growing these plants. Therefore, it is suggested that more studies be conducted on the effect of these essences over other fungi as well as animals and human disease-causing bacteria in order to gain better results in their purification and improvement of their extraction methods. Furthermore, it is also suggested that a combination of two or more essences be examined in definite cure of infections and preventing their reoccurrence. This way not only could we progress in treating diseases, but we can also become more self-sufficient in pharmaceutical and medical sciences.

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